a time scale of seconds, the decay of ΔF to resting levels was much slower in fast-twitch than slow-twitch fibers (average time constants of ~12 vs. ~2 s). This long tail of the Ca transient in fast-twitch fibers, which can last up to 60 s, is consistent with the large concentration of the calcium binding protein parvalbumin in EDL but not soleus fibers. Modeling of myoplasmic Ca movements in fast-twitch fibers indicates that, 1 s after an AP, most (~0.8) of the released Ca remains in the myoplasm where it is mainly (~95%) bound to parvalbumin. Ca binding by parvalbumin helps to abbreviate the Ca transient during relaxation. However, because parvalbumin brings free [Ca] close to resting levels, the modeled activity of the Ca pump is low. In consequence, Ca pumping continues for tens of seconds, as Ca is slowly returned to the sarcoplasmic reticulum. Supported by NIH and MDA.

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Analysis of Calcium (Ca) Transients during Excitation-Contraction Coupling in Frog Twitch Muscle Fibers

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Singly-dissected fibers were micro-injected with furaptra, a rapidly-responding Ca indicator. Resting fluorescence (F_R) and action-potential evoked changes (ΔF) were measured at 16 °C; $\Delta F/F_R$ was scaled to units of Δf_{CaD}, the change in fraction of furaptra in the Ca-bound form. Measured Δf_{CaD} was compared with Δf_{CaD} simulated with a kinetic model of the underlying myoplasmic Ca movements. During the period 30-200 ms after an action potential, simulated Δf_{CaD} decayed toward baseline more slowly than measured Δf_{CaD}. If Δf_{CaD} was simulated with a modified model that incorporated competition between Mg and Ca for occupancy of the regulatory sites on troponin (assumed dissociation constant of Mg's reaction with the regulatory sites, 2.2 mM; assumed myoplasmic free [Mg], 1 mM), good agreement with measured Δf_{CaD} was observed. The results support the conclusion that Mg, at physiological levels, competes with Ca for occupancy of the regulatory sites, as indicated in some experiments from fragmented preparations, including tension-pCa measurements in skinned fibers and biochemical studies of isolated troponin molecules. Δf_{CaD} in frog was also compared with our previous measurements and simulations of Δf_{CaD} in mouse fibers (reviewed in J. Gen. Physiol., 2012). In frog, the SR Ca release flux elicited by an action potential appears to be the sum of two components. The time course of the first component is similar to that of the entire flux waveform in both fast-and slow-twitch mouse fibers, while that of the second is several-fold slower; the fractional release amounts are ~0.85 (first component) and ~0.15 (second component). An anatomical basis for two release components in frog is the presence of both junctional and para-junctional Ca release channels, whereas the mouse fibers have only junctional channels (Felder and Franzini-Armstrong, 2002). Supported by NIH (GM 086167)

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Myoplasmic Calcium (Ca) Movements during Relaxation and Recovery of Superfast Muscle Fibers of the Toadfish Swimbladder (TSB)

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Tsb fibers produce high-frequency contractions (80-100 Hz at 16 °C) that generate the fish's cyclical mating call (~500 ms duration, ~10 s intercall interval). Our aim was to understand Ca movements during the intercall interval by measuring Ca transients elicited by action potentials (APs). Fluo-4 was micro-injected into one fiber within a dissected bundle. With one AP, the peak, time of peak, and temporal half-width of $\Delta F/F_R$ (change in indicator fluorescence divided by resting fluorescence) were ~20, ~9 ms, and ~14 ms, respectively. The remaining analysis considered the late time course of ΔF. From 1 to 60 s after cessation of stimulation, ΔF was well fitted by a double-exponential decay plus a small offset. With a 40-AP train at 83 Hz, the two exponential components had approximately equal amplitudes; the fast and slow time constants were ~2 and ~10 s. ΔF was well simulated with our kinetic model of Ca movements in tsb fibers (model 3; Rome et al., J. Physiol., 2011) extended to the 60 s time scale. With one AP, the estimated amount of SR Ca released is ~750 μM (concentration referred to the myoplasmic water volume) and $f_M(1)$ (the amount of the released Ca still in the myoplasm 1 s after cessation of stimulation) is ~630 μM, 97% of which is bound to parvalbumin. With 40 APs and a Ca release of ~5.5 mM, $f_M(1)$ is ~3.2 mM, with 92% bound to parvalbumin. The calling cycle thus appears to be constrained by Ca accumulation on parvalbumin and the slow rate of Ca pumping that ensues when parvalbumin lowers free [Ca] close to the resting level. Supported by NIH (GM 086167) and NSF (IOS-1145981).

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TRIM50 Regulates Vesicular Trafficking for Acid Secretion in Gastric Parietal Cells

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Within the human genome, there are ~80 members of the tri-partite motif (TRIM) family proteins. Our previous studies have defined MG53, a muscle-specific TRIM protein, as an essential component of the cell membrane repair machinery. Sequence homology analysis reveals a strong sequence similarity between MG53 and TRIM50 - a stomach-specific TRIM member whose biological function has yet to be defined. Our biochemical data demonstrated that TRIM50 is specifically expressed in gastric parietal cells, and predominantly localized in the tubulovesicular and canalicular membranes. In cultured cells ectopically expressing GFP-TRIM50, confocal microscopic imaging revealed dynamic movement of TRIM50-associated vesicles in a phosphoinositide 3-kinase-dependent manner. A protein overlay assay detected preferential binding of the PRY-SPRY domain from the TRIM50 C-terminal region to phosphatidylinositol species, suggesting that TRIM50 is involved in vesicular dynamics by sensing the phosphorylated state of phosphoinositol lipids. *Trim50*-knockout mice retained normal histology in the gastric mucosa but exhibited impaired secretion of gastric acid. In response to histamine, *Trim50*-knockout parietal cells generated deranged canaliculi, swollen microvilli lacking actin filaments, and excess multilamellar membrane complexes. Therefore, TRIM50 seems to play an essential role in tubulovesicular dynamics, promoting the formation of sophisticated canaliculi and microvilli during acid secretion in parietal cells.

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Effects of Substituting Tryptophan for Basic Residues in the S4 Voltage-Sensing Helices of $Ca_V1.1$

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In skeletal muscle, $Ca_V1.1$ serves dual functions as the voltage sensor for excitation-contraction (EC) coupling and as an L-type Ca^{2+} channel. It has been long established that L-type Ca^{2+} current activates at potentials ~20 mV more depolarized than intramembrane charge movement or EC coupling. We recently characterized a $Ca_V1.1$ mutation (R174W) which affects the innermost basic residue of the voltage-sensing S4 helix of Repeat I and results in malignant hyperthermia susceptibility in humans. Interestingly, R174W abolishes activation of the L-type current in response to 200 ms depolarizations without affecting the voltage dependence of intramembrane charge movement or of SR Ca^{2+} release. In this study, we have made corresponding mutations in Repeats II (K537W), III (R906W) and IV (K1245W) and expressed these mutants in *dysgenic* ($Ca_V1.1$ null) myotubes. Confocal imaging of affixed YFP tags indicated that all the mutants were correctly targeted to membrane-SR junctions. In striking contrast to the virtual loss of channel function observed with R174W, the K537W and K1245W mutants produced enormous L-type currents with peak amplitudes nearly 8-fold greater than wild-type $Ca_V1.1$ and greatly impaired deactivation. Even though these two gain of channel function mutants activated at substantially more hyperpolarized potentials (~20 mV shift), the voltage-dependence of charge movement and SR Ca^{2+} release were largely unaffected. On the other hand, R906W produced currents of similar amplitude to wild-type $Ca_V1.1$ but with a consistent ~15 mV depolarizing shift in activation. We are currently investigating the effects of the R906W mutation on the voltage dependence of charge movement and SR Ca^{2+} release in order to determine whether RIII plays a more critical role in engaging EC coupling than the other conserved Repeats of $Ca_V1.1$. Supported by NIH AR055104 (KGB) and AG038778 (RAB), and MDA176448 (KGB).

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Protein Components of the Ryanodine Receptor Complex Traffic Directly from Rough ER to Concentrate in Junctional SR

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Interactions of the junctional SR (jSR) proteins calsequestrin (CSQ2), triadin (TRD), and junctin (JCT) with the ryanodine receptor have been demonstrated in vitro as essential for proper SR Ca^{2+} release. However, the intracellular pathway(s) by which these critical proteins traffic to jSR sites remains unknown. To investigate pathways of trafficking for jSR proteins in adult